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Cross-species hybridizations *in situ* of genes associated with meat production traits in the wild pig genome

Międzygatunkowe hybrydyzacje *in situ* genów związanych z cechami użytkowości mięsnej w genomie dzika

Summary. Ghrelin (GHRL) and uncoupling proteins UCP2, UCP3 play a functional role in global energy metabolism in the body, growth and obesity as well as meat organoleptic quality. The aim of this study was to analyze homology between the regions of human chromosomes involving the encoding *loci GHRL*, *UCP2*, *UCP3* and the corresponding fragments in the wild pig (*Sus scrofa scrofa*) genome using the FISH technique. Two commercial human probes (Vysis), specific for regions of 3. and 11, pair autosomes (HSA3p25-26 and HSA11q13) were used for cross-hybridizations *in situ* with wild pig chromosomes – karyotype 36,XY,rob(15;17). The following physical locations were established – the *GHRL* gene was identified in wild pig autosomal interstitial region SSC13q31-32 whereas *UCP2* and *UCP3* genes, due to their proximity, were mapped to the same chromosome region SSC9p21-24. Cross-species *in situ* hybridizations confirmed conservation of the linkage groups and a high degree of homology of chromosome regions containing *GHRL*, *UCP2* and *UCP3 loci* in human and the domestic and wild pig genomes.

Key words: wild pig, comparative FISH mapping, cross-species hybridizations *in situ*, *GHRL*, *UCP2*, *UCP3* genes

INTRODUCTION

Ghrelin/obestatin prepropeptide GHRL and uncoupling proteins UCP2, UCP3 plays a functional role in global energy metabolism in the body, growth and obesity as well as organoleptic meat quality [Werner *et al.* 1999, Li *et al.* 2005, 2007, Rejduch *et al.* 2010]. Based on the contemporary literatures, it was shown that the ghrelin gene (*GHRL*) contributed a series of biological functions including regulation of food intake, body weight,

gastrointestinal (G1) motility, enzyme and hormone secretion, glucose release, cardiovascular functions, cell proliferation (adipocytes, hepatocytes) and reproduction in pigs [Dong *et al.* 2009]. On the other hand, *UCP2* and *UCP3 loci* protein products are known as a mitochondrial membrane transporters involved in regulation of energy metabolism [Li *et al.* 2005, 2007]. The studies of genes encoding these proteins, involving the genetic variation underlying economically important traits, may be useful for searching of markers associated with meat production in the wild pig to further integrate genomics of *Suidae* species.

The aim of this study was comparative cytogenetic mapping of *GHRL* as well as *UCP2* and *UCP3* genes in the wild pig genome (*Sus scrofa scrofa*) with the use of commercial human probes, specific for human chromosome regions HSA3p25-26 and HSA11q13-14, comprising *loci* of these genes.

MATERIAL AND METHODS

Metaphase chromosome spreads (50 cells) of wild pig with karyotype 36,XY, rob(15;17) were obtained from (pokeweed mitogen stimulated) peripheral blood lymphocyte culture, according to the standard protocol and routine karyotype analysis. Chromosome localization was performed by FISH on DAPI-FISH banded metaphase plates. International nomenclature of pig chromosomes was applied [Gustavsson 1988].

Comparative cytogenetic mapping of *GHRL*, *UCP2* and *UCP3* genes on chromosomes of the wild pig (*Sus scrofa scrofa*) was performed by FISH technique (according to the manufacture's procedure) with the use of two commercial human probes (Vysis), specific for human chromosome regions HSA3p25-26 (3PTEL25, D3S4559 Spectrum Green) and HSA11q13-14 (LSI Cyclin D1, 11q13 Spectrum Orange) comprising *loci* of these genes. Hybridization signals were observed under an Axio Imager.D2 (Zeiss) fluorescent microscope equipped with Axio Vision computer-assisted image analysis system.

RESULTS

The results presented in Figure 1 show distinct yellow fluorescence signals on SSC13 and red signals on SSC9. The following physical locations were established – the *GHRL* gene was identified in wild pig autosomal interstitial region SSC13q31-32 whereas *UCP2* and *UCP3* genes, due to their proximity, were mapped to the same chromosome region SSC9p21-24. Cross-species *in situ* hybridizations confirmed conservation of the linkage groups and high degree of homology of chromosome regions containing *GHRL, UCP2* and *UCP3 loci* in the compared species.

DISCUSSION

Genetic control of fat tissue accumulation is an important issue in pig breeding and production, due to its relationship with meat quality traits. Knowledge on the location of the porcine genes encoding proteins playing a crucial role in global energy metabolism,

growth, obesity and adipogenesis, lipid metabolism and transport as well as adipokines, is rapidly developing. Taking into account that the pig is considered a useful animal model for human obesity comparative studies on locations and mutations of candidate genes in this species are of interest [Lunney 2007, Clarke 2008]. One of the techniques developed in order to precisely identify segmental chromosome homology between humans and pigs was cross-species *in situ* hybridizations (Zoo-FISH) [Scherthan *et al.* 1994, Rettenberger *et al.* 1995, Frönicke *et al.* 1996].

The importance of the Zoo-FISH approach has been emphasized mainly for searching genomic regions and candidate loci governing traits of biological or economic importance, with increasing interest in mapping of quantitative trait loci (QTL) for growth and obesity in these species [Kim *et al.* 2004, Rothschild *et al.* 2007]. Significant progress in comparative mapping has been made by bidirectional heterologous chromosome painting, followed by somatic cell or radiation hybrid panels application, and supplemented by linkage analysis [Goureau *et al.* 1996, Frönicke *et al.* 1996, Yerle *et al.* 1996, 2002]. Construction of these well-integrated cytogenetic and genetic maps contributed to the assignment of 170 conserved chromosomal blocks or syntenic segments in genomes of these species [Milan *et al.* 2006, Chen *et al.* 2007, Jiang and Rothschild 2007, Rothschild *et al.* 2007]. Both molecular cytogenetic techniques and comparative genetic linkage maps defined complete synteny conservation between human chromosome 3 (HSA3) and pig chromosome 13 (SSC13) as well as autosome 11 (HSA) and 9 (SSC9) encompassing many conserved segments [Johansson *et al.* 1995, Milan *et al.* 1996, Chowdhary *et al.* 1998, Jiang and Rothschild 2007].

The cross-species FISH mapping with human chromosome regional probes reported in this paper proved total correspondence and homology between human and porcine chromosomal segments HSA3p26-25 and SSC13q31-32 as well as HSA11q13-14 and SSC9p21-24, containing the studied functionally important genes: *GHRL* – Ghrelin/obestatin prepropeptide; *UCP2* – uncoupling protein 2; *UCP3* – uncoupling protein 3 (according with comparative gene map databases: https://www-lgc.toulouse.inra.fr/pig/compare/HSA.htm, http://www.ncbi.nlm.nih.gov/projects/genome/guide/pig/). The above mentioned *loci* were earlier mapped on specific porcine chromosomes by somatic cell hybridization (SCH) and FISH technique with porcine BAC clone or human chromosomal region-specific probes [Werner *et al.* 1999, Nowacka-Woszuk *et al.* 2008, Rejduch *et al.* 2010] as well as only provisionally in the wild pig genome [Kozubska-Sobocińska *et al.* 2014]. All the presented localizations were in agreement with their physical positions in the human genome, based on the human-pig comparative chromosome-painting map [Goureau *et al.* 1996].

It is noteworthy that the studied genes were located within or near QTL for fatness traits. *GHRL* gene enclosed in the SSC13q31-32 region has been taken into account as a putative major gene for QTL affecting fatty acid composition, combining an increase of intramuscular fat content (IMF) enhancing monounsaturated fatty acid percentage in different pig breeds [Sanchez *et al.* 2007, Rejduch *et al.* 2010]. Location of porcine *GHRL* gene confirmed by cross-species chromosome painting described in our paper will be helpful in more accurate mapping of this QTL, which would be of great interest in the pig because IMF is defined to play a key role in organoleptic meat quality. The increase of IMF is associated with an improvement in consumer perception of texture and taste. Thus, in Large White and Landrace breeds, increasing IMF content (at least in the *Long-*

issimus dorsi muscle), is reported as highly desirable. Additionally, not only the amount of IMF has to be considered but also fatty acid composition, which is known to affect technological quality of fresh meat and sensory value of pig meat products [Sanchez *et al.* 2007]. Similarly, *UCP2* and *UCP3* genes were mapped within or near QTL for lipid content and intramuscular fat. Besides, numerous polymorphisms (SNPs and InDels) were found to be associated with body weight [Li *et al.* 2005, 2007, Nowacka-Woszuk *et al.* 2008]. Summarizing, reported in this paper, comparative analysis of obesity-related genes in wild pigs is important for development of marker-assisted selection on growth and fat deposition traits in *Suidae* species.

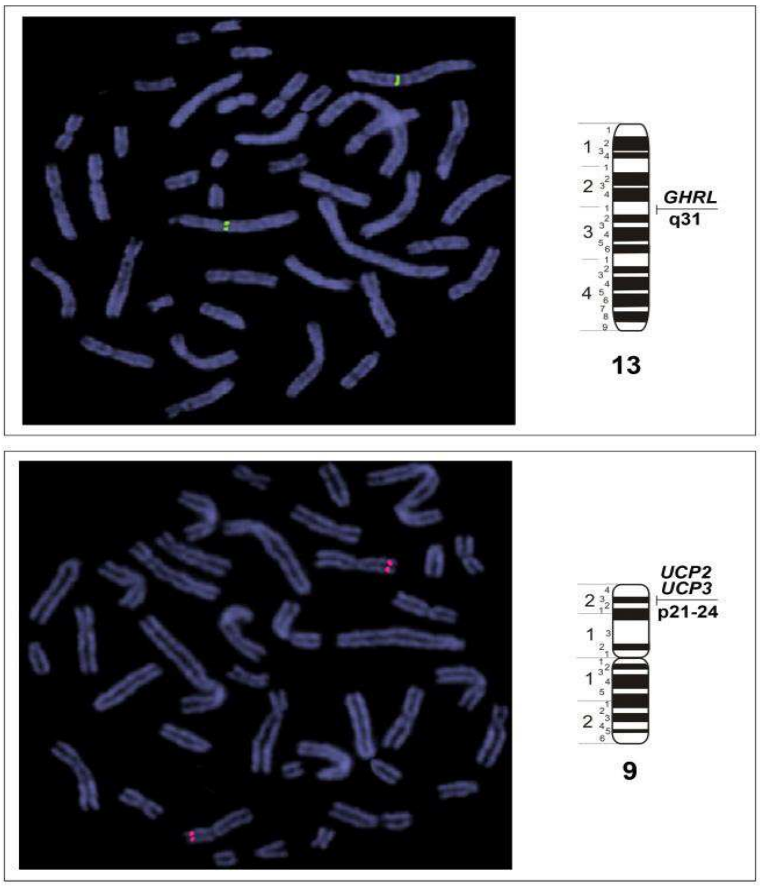

Fig. 1. The cross-species FISH mapping with human region-specific chromosome probes containing the studied *loci*. Yellow-green signal identifies *GRHL* gene in wild pig chromosomes q-arm interstitial region 13q31-32 (SSC13q31-32) (upper), red fluorescence signal labels *UCP2* and *UCP3* genes in region 9p21-24 of the wild pig genome (SSC9p21-24) (below)
Rys. 1. Międzygatunkowe mapowanie FISH ludzkimi sondami specyficznymi dla regionów chromosomowych zawierających badane *loci*. Żółty sygnał identyfikuje gen GHRL na chromosomach dzika w interstycjalnym regionie ramion q (u góry), czerwony sygnał fluorescencyjny znakuje geny UCP2 i UCP3 w regionie 9p21-24 genomu dzika (SSC9p21-24) (u dołu)

CONCLUSION

Cross-species *in situ* hybridizations confirmed conservation of the linkage groups and high degree of homology of chromosome regions containing *GHRL*, *UCP2* and *UCP3 loci* in human and the domestic and wild pig genomes.

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Streszczenie. Grelina (GHRL) i białka rozprzęgające UCP2, UCP3 pełnią funkcjonalną rolę w globalnym metabolizmie ciała, wzroście i otłuszczeniu, a także w organoleptycznej jakości mięsa. Celem badań była analiza homologii między regionami chromosomów człowieka zawierających *loci GHRL*, *UCP2*, *UCP3* a odpowiadającymi im fragmentami genomu dzika (*Sus scrofa scrofa*) przy wykorzystaniu techniki FISH. Do międzygatunkowych hybrydyzacji *in situ* z chromosomami dzika – kariotyp 36,XY,rob(15;17) – wykorzystano dwie komercyjne ludzkie sondy (Vysis), specyficzne dla regionów autosomów 3 i 11 pary (HSA3p25-26 i HSA11q13). Ustalono następujące fizyczne lokalizacje – gen *GHRL* zidentyfikowano u dzika w autosomalnym interstycjalnym regionie SSC13q31-32, a geny *UCP2* i *UCP3*, ze względu na bliskie sąsiedztwo, wymapowano w tym samym regionie chromosomu SSC9p21-24. Międzygatunkowe hybrydyzacje *in situ* potwierdziły konserwatyzm grup sprzężeniowych oraz wysoki stopień homologii regionów chromosomowych zawierających *loci GHRL*, *UCP2* i *UCP3* w genomach człowieka, świni domowej i dzika.

Słowa kluczowe: dzik, mapowanie porównawcze FISH, międzygatunkowe hybrydyzacje *in situ*, geny *GHRL*, *UCP2* i *UCP3*, cechy użytkowości mięsnej